

ALYTES

INTERNATIONAL JOURNAL OF BATRACHOLOGY

February 2011

Volume 27, N° 3

Alytes, 2011, 27 (3): 73–84.

Age profile in nine *Mantella* poison frogs from Madagascar, as revealed by skeletochronological analyses

Franco ANDREONE*, Cristina GIACOMA**, Fabio M. GUARINO***, Vincenzo MERCURIO**** & Giulia TESSA*

* Museo Regionale di Scienze Naturali,
Via G. Giolitti, 36, 10123 Torino, Italy
<franco.andreone@regione.piemonte.it>

** Dipartimento di Biologia Animale e dell'Uomo,
Università degli Studi di Torino,
Via A. Albertina, 13,
10123 Torino, Italy
<crisrina.giacoma@unito.it>

*** Università degli Studi di Napoli,
Dipartimento di Biologia Strutturale e Funzionale,
Via Cintia, 80126 Napoli, Italy
<fabio.guarino@unina.it>

**** Museum für Naturkunde,
Leibniz-Institut für Evolutions- und Biodiversitätsforschung
an der Humboldt-Universität zu Berlin,
Invalidenstraße 43,
10115 Berlin, Germany
<vincenzomercurio@gmx.de>

Skeletochronology has been successfully used to age temperate amphibians, enabling comparisons of longevity and the age at sexual maturity. To date, however, there have been few similar studies conducted using this technique in tropical amphibians. Here we present data on age structure and age at maturity for nine species of Malagasy *Mantella* frogs: *M. baroni*, *M. bernhardi*, *M. sp. aff. expectata*, *M. cowani*, *M. crocea*, *M. laevigata*, *M. nigricans*, *M. pulchra* and *M. viridis*. The genus *Mantella* includes some of the most threatened frog species in Madagascar, and also some of the most requested species for the international pet trade. The lack of basic information on the life history of these species in the wild is hindering the development of sustainable collection models. We analysed museum specimens and bone samples of free living individuals collected during several surveys in western and eastern Madagascar. All investigated species showed a comparatively short longevity (0-4 LAGs) and sexual maturity was reached on the 1st or the 2nd year of life.

INTRODUCTION

The island of Madagascar is one of the most extraordinary amphibian hot-spots in the World: there are reliable estimated to be as many as 465 species, and many new taxa are being



described at a very rapid rate (ANDREONE et al., 2008; VIEITES et al., 2009). Even though major progress has been made in describing the diversity of the Malagasy amphibian fauna, much still remains to be understood about the ecology and life history traits of most species, and about the practical implications for conservation.

One of the best known frogs of Madagascar are those belonging to the genus *Mantella*, the Malagasy poison frogs. Currently, 16 species have been already described, and a few others still wait for a formal description (GLAW & VENCES, 2007). Their fame is due mainly to their bright aposematic colouration, their diurnal behaviour and the relative facility to keep them in captivity (at least for some species), that make them among the most sought after frogs for the international pet-trade (ANDREONE et al., 2006; GLAW & VENCES, 2007). So far, it is commonly accepted that many *Mantella* species are threatened because of a combination of narrow distribution, increasing deforestation and habitat degradation and intensive collection (ANDREONE et al., 2005a). The most recent conservation assessment (ANDREONE et al., 2008; ANONYMOUS, 2009) highlighted three species (*M. aurantiaca*, *M. cowani* and *M. milotympanum*) as "Critically Endangered", and four as "Endangered" (*M. bernhardi*, *M. crocea*, *M. expectata* and *M. viridis*).

Whether the pet-trade could be a primary cause of conservation concern and population/species decrease for amphibians is still matter of controversy and is worth of a more in-depth investigations in the future. The impact of collection from the wild on most *Mantella* species is still poorly understood and information on the breeding biology of species is badly needed. The whole genus *Mantella* is now listed on CITES II (ANDREONE et al., 2005a, 2006), and the trade is managed through a system of annual quotas (TESSA et al., 2009). In terms of commercial exploitation, a review carried by RABEMANANJARA et al. (2008b) reported a total of about 230,000 individuals exported from Madagascar between 1994 and 2003. Although this number is quite low when compared to around two million *Hymenochirus* and 1.6 million *Cynops orientalis* referred to the importation trade for the USA in 1998–2002 (SCHLAEPFER et al., 2005), it still represents a considerable portion of traded amphibians, capable of generating important economic benefits.

Seen all these aspects, it is our conviction that a special attention should be paid to gathering life history data for most *Mantella* species, in order to assess a better comprehension of the threats affecting the species. Population estimates, fecundity and age structure are among the most important factors to be taken into consideration, and could help in drawing the conservation profiles of the traded species.

Despite the commercial interest in the genus, there is little information on *Mantella* life history traits in the wild (VENCES et al., 1999). Scattered information on longevity of Malagasy frogs (especially mantellas and other traded species) are available from observations from captive individuals published in herpetoculturist papers and books (e.g., STANISZEWSKI, 2001), or as web-literature (CAREY & JUDGE, 2000). So far, the value in terms of conservation utilisation of these data is highly questionable. First of all because they are often reported in a non systematic way, then because the husbandry conditions are usually very different from what is the reality in nature. For this reason we do not consider these data as indicative of the real maximum longevity of a species.

A robust method used to assess longevity is skeletochronology (CASTANET, 1975). It has been widely used for individual age determination (ANDREONE et al., 2002, 2005b; GUARINO et

al., 1998, 2008; TESSA et al., 2007; KUMBAR & PANCHARATNA, 2001). Here we report skeletochronological data of nine *Mantella* species which significantly add to information already available (GUARINO et al., 2008), and verify the congruence between maximum longevity and age at sexual maturity.

MATERIAL AND METHODS

We analysed bone samples of the following *Mantella* species: *M. baroni* (15 males and 9 females; data from GUARINO et al., 2008); *M. bernhardi* (20 males and 12 females); *M. sp. aff. expectata* (9 males and 6 females); *M. cowani* (14 males and 12 females; data from GUARINO et al., 2008); *M. crocea* (13 males and 1 female); *M. laevigata* (5 males and 5 females); *M. nigricans* (5 males and 5 females); *M. pulchra* (13 males, 12 females); and *M. viridis* (20 males and 20 females). A complete list of the analysed specimens and their provenance is given in tab. 1 and in app. 1. The individuals from the Isalo population, attributed to *M. betsileo* by CROTTINI et al. (2008), are here considered as a still undescribed species, *M. sp. aff. expectata* "South", according to GLAW & VENCES (2007). The individuals attributed to *M. crocea* populations (according to GLAW & VENCES 2007) were considered as *M. cf. milotympanum* by BORA et al. (2008).

Frogs were sampled during the rainy season (October-March), when they are active and show breeding habits. They were localized by sight and by acoustic emissions of males. Once captured, they were sexed (males are usually smaller than females and often show femoral glands, or have differential chromatic characters; see JOVANOVIĆ et al., 2006), and measured for snout-vent length (SVL, at the nearest 0.1 mm). For one species, *M. bernhardi*, the study was partly conducted on phalanges taken from wild (non-captured) individuals and partly from preserved specimens. In such a case, the third toe of each captured individual was cut in the field, put in 90 % ethanol and then processed for skeletochronology and genetical analysis. After toe-clipping, the specimens were released at the site of capture or conserved as museum voucher specimens. For all the other species, the phalanx was taken directly from preserved voucher specimens, now part of the herpetological collections of Museo Regionale di Scienze Naturali, Torino, Italy (MRSN), and Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar (PBZT).

In *M. bernhardi*, we analysed separately individuals of two different populations which differed for habitat conditions: the first one from a rather intact rainforest site (Mangevo) within the Parc National de Ranomafana, and the second one from a heavily deforested site (Ambohimandrozo) next to the presumed species' type locality (RABEMANANJARA et al., 2005; VIEITES et al., 2006).

The skeletochronological method followed the protocol used for other Malagasy amphibians (GUARINO et al., 2008). Phalanges were decalcified in 3 % nitric acid for 1 hour, sectioned at 12 μ m and stained in Ehrlich's haematoxylin for about 15 minutes. Finally, two researchers observed independently the sections and counted the LAGs, using a light microscope. In individuals sampled at the beginning of the rainy season (just after the latency period), the last LAG is positioned close (sometimes coincident) to the external edge of the

Table 1. – Data on provenance of samples of species of the genus *Mantella* analysed by skeletochronology.

Species	Sites	Coordinates	Altitude m a.s.l.	Habitat	Period of collection
<i>Mantella baroni</i>	Antoetra	20°50.10' S 47°19.95' E	~ 1400	Montane fragmented rainforest	I-II.2003
<i>Mantella bernhardi</i>	Ambohimandrozo	21°28.43' S 47°33.37' E	~ 600	Secondary altered rainforest	I-II.2004
<i>Mantella bernhardi</i>	Mangevo	21°23.25' S 47°27.38' E	~ 500	Rainforest	I-II.2004
<i>Mantella cowani</i>	Antoetra	20°50.10' S 47°19.95' E	~ 1400	Montane fragmented rainforest	I-II.2003
<i>Mantella</i> sp. aff. <i>expectata</i>	Isalo	22°37.38' S 45°21.52' E	~ 800	Savannah, dry forest	XI-XII.2004
<i>Mantella crocea</i>	Fierenana	18°34.36' S 48°26.38' E	~ 910	Rainforest	XII.2003
<i>Mantella laevigata</i>	Masoala	15°18.01' S 50°03.09' E	~ 615	Rainforest	XI-XII.1998-1999
<i>Mantella nigricans</i>	Masoala	15°18.01' S 50°03.09' E	~ 615	Rainforest	XI-XII.1998-1999
<i>Mantella pulchra</i>	Fierenana	18°34.36' S 48°26.38' E	~ 910	Rainforest	I.1999
<i>Mantella viridis</i>	Antongombato	12°23 03' S 49°13 27' E	~120	Secondary forests, dry forest	I.2005

bone section because the maximal growth season has not yet started. In such cases we considered the external border of the section as a LAG itself.

Then, in order to estimate age at sexual maturity, we followed the criterion of LAG rapprochement as proposed by KLEINEBERG & SMIRINA (1969) and widely used by other authors (e.g., FRANCILLON-VIEILLOT et al., 1990; LÉCLAIR et al., 2005; TSIORA & KYRIAKOPOULOU-SKLAVOUNOU, 2002). Therefore, the first decreasing interval between LAGs, which is supposed to indicate the attainment of sexual maturity, was identified for each section.

All numerical data were analyzed by Student's *t*-test. A probability level of $P \leq 0.05$ was considered as significant. We compared the values (mean \pm standard deviation) of SVL, maximum LAG number and number of LAGs at the sexual maturity for males and females whose samples were greater than five individuals.

RESULTS

At a qualitative analysis the bone sections of all the studied species were similar and composed of two concentric layers, which corresponded to the endosteal (the innermost) and periosteal (the outermost) bone (Fig. 1). In the periosteal bone, we noticed contrasted haematoxyphilic lines that were considered as reliable LAGs. In some individuals, similar

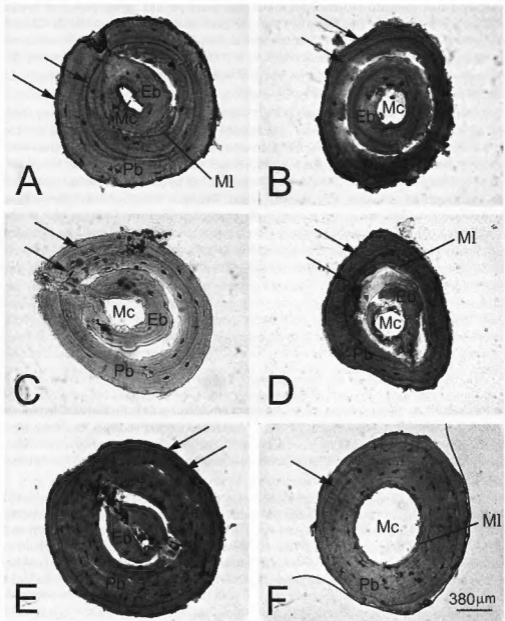


Fig. 1. – Histological section in the analysed *Mantella* species. Arrows indicate the lines of arrested growth (LAGs). A: *M. bernhardi*, MRSN A3112, female with 2 LAGs, SVL = 21.8 mm; B: *M. sp. aff. expectata*, MRSN A5234, male with 2 LAGs, SVL = 21.1 mm; C: *M. laevigata*, MRSN A4531, male with 2 LAGs, SVL = 24.8 mm; D: *M. pulchra*, MRSN A3060, female with 2 LAGs, SVL = 26.1 mm; E: *M. viridis*, MRSN A5082, female with 2 LAGs, SVL = 32.0 mm; F: *M. viridis*, MRSN A5112, male with 1 LAGs, SVL = 25.1 mm. Abbreviations: Eb, endosteal bone; Mc, medullary cavity; MI, metamorphosis line; Pb, periosteal bone.

lines were also visible in endosteal bone and thus corresponded to the periosteal LAGs. Although a reversal line (the boundary between periosteal and endosteal bone) was not always easy to detect, we assumed that the bone erosion did not delete any LAGs, assuming the short longevity of these frogs. In addition, in 47.5% of the specimens we detected the presence of the metamorphosis line, a line visible near the reversal line occurring at the passage from tadpoles to metamorphosed froglets.

All the species were small bodied (SVL 13-33 mm) and showed a comparatively short life span (0-4 LAGs). Females were significantly larger than males, excepting for the *M. bernhardi* population from Ambohimandrozo. In six species the mean LAG values were higher in females than in males, but these differences were significant only for *M. cowani*. Males and females reached sexual maturity the first or, less frequently, the second year, in all the species (mean \pm SD = 1.37 ± 0.31 years). The only exception was observed for *M. laevigata*, in which the sexual maturity was attained at the second year in nine of the 10 individuals; in this species the longevity was two years, and thus sexual maturity coincided with the life expectancy. In all the species except for *M. pulchra*, males reached sexual maturity earlier than females, but the only species in which this difference was significant were *M. aff. expectata* and *M. viridis*.

DISCUSSION

The data here presented represent the most exhaustive contribution on skeletochronology applied to a single tropical amphibian genus. Moreover, as stressed before, most of the analysed *Mantella* specimens came from series held in collections of natural history museums. So far, we advocate the importance of using preserved museum vouchers as a long-term source of biological data, and this is not only useful, but also relevant in conservation terms, since it maximises the amount of information that can be obtained from zoological collections (TESSA et al., 2009).

Our data also confirm that skeletochronology is reliable and successful for tropical amphibians, despite of their provenience and ecology. All the studied species showed evident LAGs, thus indicating that they observed a period of growth and a period of inactivity and latency, as this was witnessed by the differential bone deposition and different chromophily in bone sections. Although we cannot provide definitive conclusions about the life history of the species because of the small sampled numbers, we noticed that all of the analysed frogs had a short life span, and this could be put in relation with their small body size. Skeletochronological studies on anurans utilizing both sexes show that in most species there is a positive correlation between body size and age both in males and females (RYSER, 1988; ESTEBAN et al., 1996, 1999). Moreover, there is often a wide size overlap among age classes even if body length and age are positively correlated.

Our results for nine *Mantella* species are consistent with longevity patterns observed in other amphibians of Madagascar, such as *Boehmantis microtympanum* (GUARINO et al., 1998), *Dyscophus antongilii* (TESSA et al., 2007) and *Boophis tsilomaro* (ANDREONE et al., 2002). These species are much larger than mantellas, all exceeding 80 mm (with a body size

record in *D. antongilii* of 110 mm), and reach a maximum age of 11 years (in *B. microtypanum* and *B. tsilomaro*). In the *Mantella* species, the body miniaturisation, which is associated to mirmecophagy, diurnal activity, and poison segregation at skin level (see VENCES et al., 1998), is a limit for their maximum life expectancy that does not exceed four years in the wild.

In six species, males had a shorter longevity than females, but this difference was significant only for *M. cowani*. This population comes from a high plateau site at around 1400 m a.s.l. (GUARINO et al., 2008). In particular, males of this species showed short mean longevity, with 1.2 ± 0.2 LAGs, while females had 2.2 ± 0.2 LAGs. So far, we assume that females can effectively live more than males, and reach a larger body size, as shown in tab. 2. We do not have sound explanations for such a difference, but we may hypothesise that females carry out a much more hidden life, and thus are less subject to predation, this leading to a differential mortality between the sexes.

We also presented information about the age at sexual maturity from skeletochronology. This was reached quite early (< 1–2 years) in all the examined species, except for *M. laevigata*. This arboreal species (GLAW & VENCES, 2007) showed a different age profile, with sexual maturity reached at two years in all the examined individuals. Unfortunately, our sample was too small to draw such a significant explanatory hypothesis, but, probably, in this species the majority of the individuals reproduce after two years of age and possibly die just after. Most likely, the delay in reaching sexual maturity could allow to get a larger size that can be useful in males for the male-male fights over defended resources necessary for reproductive success and in females for maternal care (HEYING, 2001). More studies are needed on this species to understand whether this difference is constant and which could actually be the advantages for both sexes.

In general, males reach sexual maturity earlier than or at the same age as females, but the only significant differences were found in *M. sp. aff. expectata* (1.2 ± 0.1 versus 1.3 ± 0.2 LAGs) and in *M. viridis* (1.0 ± 0.0 versus 1.1 ± 0.1 LAGs). Notably, these are the only two species from open grassland habitats, with accentuated seasonality (TESSA et al., 2009).

In several anuran species, the sexes reach sexual maturity at the same age (ESTEBAN et al., 2004), whereas in others females reach sexual maturity later than males (CHERRY & FRANCILLON, 1992). These data may be explained by the fact that females start breeding when they reach a minimum body size to maximize clutch mass (CHERRY & FRANCILLON, 1992; ESTEBAN et al., 1996). On the other hand, in anuran females delayed maturity often means a larger body size and higher fecundity (GIBBONS & MCCARTHY, 1984), and sexual selection for larger body size in females occurs when it represents an important determinant of female reproductive success.

The age profile can also provide information on the conservation status of the studied species, as witnessed by the two studied *M. bernhardi* populations, which showed a different LAG number profile. The individuals from Mangevo, which is a rather intact rainforest, showed a comparatively larger body size and a longer life than those from the increasingly deforested site of Ambohimandrozo (SVL: males: $t = 1.88$, $P < 0.05$; females: $t = 4.44$, $P < 0.05$; LAGs: males: $t = 1.05$, $P < 0.05$; females: $t = 2.03$, $P > 0.05$). Instability of habitat quality and the intense degradation following anthropogenic may be invoked as a cause to decrease population viability.

Table 2. – Data on body size (SVL in mm), longevity (LAG numbers) and age at sexual maturity (given as LAG numbers, largely corresponding to years) in the analysed species. Values are provided as mean \pm standard deviation. The numbers between round brackets refer to the number of analysed specimens, whereas the values between square brackets are the extreme range values; *t*, Student's *t* value; asterisked values are significantly different for $P < 0.05$ (*) or for $P < 0.01$ (**).

Species	Sex	SVL	<i>t</i>	Maximum number of LAGs	<i>t</i>	LAGs at sexual maturity	<i>t</i>
<i>Mantella baroni</i>	Males (15)	25.6 \pm 2.4 [18.6-28.5]	3.48**	4.6 \pm 0.2 [1-3]	1.07	1. \pm 0.5 [1-2]	0.09
	Females (9)	28.6 \pm 1.2 [26.9-30.1]		1.9 \pm 0.1 [1-2]		1.4 \pm 0.5 [1-2]	
<i>Mantella bernhardi</i> (Mangevo)	Males (10)	20.1 \pm 0.8 [19.1-21.8]	-	1.8 \pm 0.6 [1-3]	-	1.3 \pm 0.5 [1-2]	-
	Females (2)	23.2 \pm 0.7 [22.6-23.8]		2.5 \pm 0.7 [2-3]		2.0 \pm 0.0 [2]	
<i>Mantella bernhardi</i> (Ambohimandrozo)	Males (10)	19.3 \pm 1.1 [17.1-20.5]	1.41	1.2 \pm 0.8 [1-3]	0.98	1.0 \pm 0.0 [1]	0.17
	Females (10)	20.0 \pm 1.2 [18.1-21.8]		1.5 \pm 0.9 [1-3]		1.1 \pm 0.3 [1-2]	
<i>Mantella cowani</i>	Males (14)	25.7 \pm 1.7 [15.1-28.9]	6.29**	1.3 \pm 0.2 [1-2]	7.86**	1.0 \pm 0.4 [1-2]	2.01
	Females (12)	29.3 \pm 1.1 [27.9-31.4]		2.2 \pm 0.2 [1-3]		1.3 \pm 0.3 [1-2]	
<i>Mantella crocea</i>	Males (13)	17.2 \pm 1.1 [14.8-19.5]	-	2.8 \pm 0.7 [2-4]	-	1.2 \pm 0.1 [1-2]	-
	Female (1)	21.6 -		2 [2]		2.0 [2]	
<i>Mantella</i> sp. aff. <i>expectata</i>	Males (9)	20.9 \pm 1.5 [18.7-23.3]	4.53**	1.9 \pm 0.8 [1-3]	0.26	1.2 \pm 0.1 [1-2]	2.36*
	Females (6)	24.0 \pm 1.0 [23.0-25.3]		1.8 \pm 0.5 [1-2]		1.3 \pm 0.2 [1-2]	
<i>Mantella lucvigata</i>	Males (5)	24.2 \pm 0.5 [23.7-24.8]	4.01**	2.0 \pm 0.0 [2]	0.99	2.0 \pm 0.0 [2]	0.00
	Females (5)	26.2 \pm 1.0 [24.6-27.0]		1.8 \pm 0.5 [1-2]		2.0 \pm 0.0 [2]	
<i>Mantella nigricans</i>	Males (5)	25.1 \pm 0.9 [24.1-26.0]	4.06**	2.8 \pm 0.8 [2-4]	1.13	1.2 \pm 0.1 [1-2]	0.00
	Females (5)	27.2 \pm 0.7 [26.5-28.3]		1.8 \pm 0.8 [1-3]		1.2 \pm 0.1 [1-2]	
<i>Mantella pulchra</i>	Males (13)	20.6 \pm 2.1 [18.4-22.7]	3.63**	1.9 \pm 0.7 [1-3]	0.73	1.6 \pm 0.1 [1-2]	1.07
	Females (12)	23.6 \pm 2.0 [21.3-28.2]		2.1 \pm 0.9 [1-4]		1.5 \pm 0.2 [1-2]	
<i>Mantella viridis</i>	Males (20)	25.9 \pm 2.1 [23.1-31.2]	8.42**	1.3 \pm 0.6 [1-3]	1.43	1.0 \pm 0.0 [1]	4.97**
	Females (20)	30.4 \pm 1.2 [28.5-32.5]		1.6 \pm 0.7 [1-3]		1.1 \pm 0.1 [1-2]	

Our final consideration goes to the advantage of putting together a series of integrative data for conservation purposes. In particular, for the genus *Mantella*, the data on age structure, longevity and age at sexual maturity here presented represent a crucial complement to those on fecundity, distribution and population consistency (e.g., RABEMAMANJARA et al., 2008a; TESSA et al., 2009). All these parameters could be usefully utilised to test extinction probability of species and populations associated to the different threat causes using population viability analyses. In particular, by comparing the survival probability of threatened

species in relation to different pet-trade collection pressures may provide indications about the sampling limit for national and international trade. On the occasion of future works, longevity data should be integrated with data on the type and the state of the habitat, and the fragmentation and the density of each harvested population, so to implement tailored conservation programs.

ACKNOWLEDGEMENTS

We are indebted to the Parc Botanique et Zoologique de Tsimbazaza and the Direction des Eaux et Forêts for permissions to visit the protected areas and for collecting the preserved animals. The fieldwork was financially supported by DAPTF, Amphibian Specialist Group, Acquario di Genova, Madagascar Fauna Group, Nando Peretti Foundation, Gondwana Conservation and Research, Van Thienhoven Foundation, and Wildcare Institute. Thanks to two anonymous referees for their useful comments.

LITERATURE CITED

- ANONYMOUS [IUCN]. 2009. - *IUCN Red list of threatened species*. Version 2009.2. <<http://www.iucnredlist.org>>.
- ANDREONE, F., CADLE, J. E., COX, N., GLAW, F., NUSSBAUM, R. A., RAXWORTHY, C. J., STUART, S. N., VALLAN, D. & VENCES, M., 2005a - Species review of amphibian extinction risks in Madagascar conclusions from the Global Amphibian Assessment *Conservation Biology*, **19**: 1790-1802.
- ANDREONE, F., CARPENTER, A. I., COX, N., DU PREEZ, L., FREEMAN, K., FLURRER, S., GARCÍA, G., GLAW, F., GLOS, J., KNOX, D., KÖHLER, J., MENDELSON III, J. R., MERCURIO, V., MITTERMEIER, R. A., MOORE, R. D., RABISOA, N. H. C., RANDRIAMAHAZO, H., RANDRIANASOLO, H., RASOAMAMPIONONA RAMINOSOA, N., RAVDAHANGIMALALA RAMILJAONA, O., RAXWORTHY, C. J., VALLAN, D., VENCES, M., VIEITES, D. R. & WELDON, C., 2008 - The challenge of conserving amphibian megadiversity in Madagascar. *PLoS Biology*, **6** (5): 943-946.
- ANDREONE, F., GUARINO, F. M. & RANDRIANIRINA, J. E., 2005b. Life history traits, age profile and conservation biology of the panther chameleon, *Furcifer pardalis* (Cuvier 1829) at Nosy Be, NW Madagascar. *Tropical Zoology*, **18**: 209-225.
- ANDREONE, F., MERCURIO, V. & MATTIOLI, F., 2006 - Between environmental degradation and international pet trade: conservation strategies for the threatened amphibians of Madagascar. *Natura Società italiana di Scienze naturali e Museo civico di Storia naturale di Milano*, **95** (2): 67-80.
- ANDREONE, F., VENCES, M., GUARINO, F. M. & RANDRIANIRINA, J. E., 2002. Natural history and larval morphology of *Boophis occidentalis* (Anura, Mantellidae: Boophinae) provide new insights into the phylogeny and adaptive radiation of endemic Malagasy frog. *Journal of Zoology* **257**: 425-438.
- BORA, P., DOLCH, R., JENKINS, R., JOVANOVIĆ, O., RABIMANJARA, F. C. E., RANDRIANIRINA, J., RAFANOMEZANTSOA, J., RAHARIVOLOLOINAINA, L., RAMILJAONA, O., RAMINOSOA, N., RANDRIANAVELONA, R., RASELIMANANA, A., RAZAFIMAHATRATRA, B., RAZAFINDRAIBE, T. & VENCES, M., 2008 - Geographical distribution of three species of Malagasy poison frogs of high conservation priority: *Mantella aurantiaca*, *M. crocea* and *M. milotympanum* *Herpetology Notes*, **1**: 39-48.
- CARLY, J. R. & JUDGE, D. S., 2000. Longevity records life spans of mammals, birds, amphibians, reptiles, and fish. *Monographs on Population Aging*, Odense, Odense University Press, **8** (online book with unnumbered pages).
- CASTANET, J., 1975. - Quelques observations sur la présence et la structure des marques squelettiques de croissance chez les amphibiens. *Bulletin de la Société zoologique de France*, **100**: 603-620.

- CHERRY, M. I. & FRANCILLON, M. H., 1992. – Body size, age and reproduction in the leopard toad, *Bufo pardalis* *Journal of Zoology*, **228** 41–50.
- CROTTINI, A., CHIARI, Y., MERCURIO, V., MLYER, A., VENCES, M. & ANDREONE, F., 2008. – Into the canyons the phylogeography of the Malagasy frogs *Mantella expectata* and *Scaphiophryne gottlebei* in the arid Isalo Massif and its significance for conservation (Amphibia: Mantellidae and Microhylidae) *Organisms, Diversity & Evolution*, **8** 368–377.
- ESTEBAN, M., GARCIA-PARIS, M., BUCKLEY, D. & CASTANET, J., 1999. – Bone growth and age in *Rana saharica*, a water frog living in a desert environment *Annales zoologici Fennici*, **36** 53–62.
- ESTEBAN, M., GARCIA-PARIS, G. & CASTANET, J., 1996. Use of bone histology in estimating the age of frogs (*Rana perezi*) from a warm temperate climate area *Canadian Journal of Zoology*, **74**: 1914–1921.
- ESTEBAN, M., SÁNCHEZ-HERRÁIZ, M. J., BARBADILLO L. J. & CASTANET, J., 2004. – Age structure and growth in an isolated population of *Pelodytes punctatus* in northern Spain *Journal of natural History*, **38** 2789–2801.
- FRANCILLON-VIHILLOT, M. H., ARNTZEN, J. W. & GERALDIE, J., 1990. – Age, growth and longevity of sympatric *Triturus cristatus*, *T. marmoratus* and their hybrids (Amphibia, Urodela). *Journal of Herpetology*, **24**: 13–22.
- GIBBONS, M. M. & MCCARTHY, T. K., 1984. – Growth maturation and survival of frogs *Rana temporaria* L. *Holarctic Ecology*, **7**: 419–427.
- GLAW, F. & VENCES, M., 2007. *A fieldguide to the amphibians and reptiles of Madagascar* Third edition Cologne, Vences and Glaw Verlag: 1–496.
- GUARINO, F. M., ANDREONE, F. & ANGELINI, F., 1998. Growth and longevity by skeletochronological analysis in *Mantidactylus microtypanum*, a rain-forest anuran of southern Madagascar. *Copeia*, **1998** 194–198.
- GLARINO, F. M., SACCO, M., TESSA, G. & ANDRILONE, F., 2008. – Short life span of two charismatic *Mantella* species: age-structure in the critically endangered *M. cowani* and in the syntopic *M. baroni*. In F. ANDRILONE (ed.), *A conservation strategy for the Amphibians of Madagascar*, Torino, *Monografie del Museo Regionale di Scienze Naturali di Torino*, **41**: 265–276.
- HIYING, H., 2001. Social and reproductive behaviour in the Madagascan poison frog, *Mantella laevigata*, with comparisons to the dendrobatids. *Animal Behaviour*, **61** 567–577.
- JOVANOVIC, O., RABEMANANJARA, F., RAMILJAONA, O., ANDREONE, F., GLAW, F. & VENCES, M., 2006. *Frogs of Madagascar genus Mantella* Pocket Identification Guide. Washington, Conservation International (unnumbered leaflet).
- KLIJNBERG, S. E. & SMIRINA, E. M., 1969. A contribution to the method of age determination in amphibians. *Zoologicheskij Zhurnal*, **48**: 1090–1094.
- KUMBAR, S. M. & PANCHARATNA, K., 2001. Occurrence of growth mark in the cross section of phalanges and long bones of limbs in tropical anurans. *Herpetological Review*, **32**: 165–167.
- LECLAIR, M. H., LECLAIR, R. JR & GALLANT, J., 2005. Application of skeletochronology to a population of *Pelobates cultripes* (Anura: Pelobatidae) from Portugal. *Journal of Herpetology*, **39**, 199–205.
- RABEMANANJARA, F., BORA, P., CADLE, J. E., ANDRILONE, F., RAJRIARISON, E., TALATA, P., GLAW, F., VENCES, M. & VILITES, D. R., 2005. New records, potential distribution and conservation of *Mantella bernhardi*, an endangered frog species from south-eastern Madagascar. *Oryx*, **39** (3) 339–342.
- RABEMANANJARA, F., BORA, P., RAZAFINDRABI, T., RANDRIAMITSO, E., RAVOAHANGIMALALA RAMILJAONA, O., RASOAMAMPIONONA RAMINOSOA, N., RAKOTONDRAVONY, D., VIEITES, D. R. & VENCES, M., 2008a. Rapid assessment of population sizes in ten species of Malagasy poison frogs, genus *Mantella*. In F. ANDRILONE (ed.), *A conservation strategy for the Amphibians of Madagascar*, Torino, *Monografie del Museo regionale di Scienze naturali di Torino*, **41**: 253–264.
- RABEMANANJARA, F., C. E., RASOAMAMPIONONA RAMINOSOA, N., RAVOAHANGIMALALA RAMILJAONA, O., ANDRILONE, F., BORA, P., CARPENTER, A. I., GLAW, F., RAZAFINDRABI, T., VALLAN, D., VIEITES, D. R. & VENCES, M., 2008b. Malagasy poison frogs in the pet trade: a survey of levels of exploitation of species in the genus *Mantella*. In F. ANDRILONE (ed.), *A conservation strategy for the Amphibians of Madagascar*, Torino, *Monografie del Museo regionale di Scienze naturali di Torino*, **41** 277–300.
- RYSER, J., 1988. Determination of growth and maturation in the common frog *Rana temporaria*, by skeletochronology *Journal of Zoology*, **216** 673–685.

- SCHLAEPFER, M. A., HOOVER, C. & DODD, C. K., Jr, 2005 – Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. *BioScience*, **55** (3), 256–264
- STANISZEWSKI, M., 2001. *Mantellas*. Frankfurt, Chimaira 1–229.
- TESSA, G., GUARINO, F. M., GIACOMA, C., MATTIOLI, F. & ANDREONE, F., 2007 – Longevity and body size in three populations of *Dyscophus antongilu* (Anura, Microhylidae), the tomato frog from north-eastern Madagascar. *Acta herpetologica*, **2** (2): 139–146
- TESSA, G., MATTIOLI, F., MERCURIO, V. & ANDREONE, F., 2009 – Egg numbers and fecundity traits in nine species of *Mantella* poison frogs from arid grasslands and rainforests of Madagascar (Anura Mantellidae). *Madagascar Conservation & Development*, **4** (2): 113–119.
- TSIORA, A. & KYRIAKOPOULOU-SKLAVOUNOU, P., 2002 – A skeletochronological study of age and growth in relation to adult size in the water frog *Rana epevotica*. *Zoology*, **105** 55–60
- VENCES, M., GLAW, F. & BÖHME, W., 1998. Evolutionary correlates of microphagy in alkaloid-containing frogs (Amphibia Anura). *Zoologischer Anzeiger*, **236**: 217–230.
- 1999. – A review of the genus *Mantella* (Anura, Ranidae, Mantellidae) taxonomy, distribution and conservation of Malagasy poison frogs. *Alytes*, **17** (1–2): 3–72
- VILTES, D. R., CHIARI, Y., VENCES, M., ANDREONE, F., RABEMANJARA, F., BORA, P., NIETO-ROMAN, S. & MEYER, A., 2006 – Mitochondrial evidence for distinct phylogeographic units in the endangered Malagasy poison frog *Mantella bernhardi*. *Molecular Ecology*, **15**: 1617–1625.
- VIEITES, D. R., WOLLENBERG, K. C., ANDREONE, F., KÖHLER, J., GLAW, F. & VENCES, M., 2009 – Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the national Academy of Science of the U.S.A.*, **106** (20), 8267–8272.

Corresponding editor: Stéphane GROSJEAN.

APPENDIX I

LIST OF *MANTELLA* SPECIMENS USED FOR SKELETOCHRONOLOGICAL ANALYSIS

Abbreviations: MRSN, specimens housed in the Museo Regionale di Scienze Naturali, Torino, PBZT, specimens housed in the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo; Prov., Province; AA, surroundings of Antongombato and Montagne des Français, IM, Isalo Massif, MP, Masoala Peninsula

Mantella bernhardi Vences, Glaw, Peyrieras, Böhme & Busse, 1994 7 unnumbered phalanges collected from wild individuals (Mangevo, Fianarantsoa Prov., 8 II 2004); MRSN A3112-3116 (Mangevo, Fianarantsoa Prov., 8 II.2004); MRSN A3042, 12 phalanges, PBZT (two unlabelled specimens, Ambohimandrozo, Fianarantsoa Prov., 15 II 2004)

Mantella sp. aff. *expectata* Busse & Böhme, 1992 MRSN A5232-5233, A5325 (Antoha, IM, Fianarantsoa Prov., 3.XII 2004), MRSN A5223, A5225, A5228-5229, A5231, A5234-5236, A5238 (Tsiormia, IM, Fianarantsoa Prov., 3 XII.2004); MRSN A5237, A5239 (Kazofoty, IM, Fianarantsoa Prov., 2.XII.2004); MRSN A5230 (Sakavato, IM, Fianarantsoa Prov., 6.XII.2004).

Mantella crocea Pintak & Böhme, 1990. PBZT unlabelled, likely Fierenana, Toamasina Prov

Mantella laevisgata Methuen & Hewitt, 1913. MRSN A4475 (Antsarahana'Ambararato, MP, Antsiranana Prov., 4 XII.1999), MRSN A4533, A3000 (Andasin' Governera, MP, Antsiranana Prov., 6 XII 1998), MRSN A4531-4532 (Andasin' Governera, MP, Antsiranana Prov., 7 XII.1998); MRSN A3001-3002 (Andasin' Governera, MP, Antsiranana Prov., 9 XII 1998), MRSN A2999 (Behanjada, MP, Antsiranana Prov., 22 XI.1999); MRSN A4505-4506 (Menamalona, MP, Antsiranana Prov., 11.XII.1999)

Mantella nigricans Guibé, 1978 MRSN A4457 (Andasin' Governera, MP, Antsiranana Prov., 5 XII 1998), MRSN A4467 (Ambatoledama, MP, Antsiranana Prov., 17 XI 1998); MRSN A4454 (Ambatoledama, MP, Antsiranana Prov., 16 XI 1998); MRSN A4508 (Antsarahana'Ambararato, MP,

Antsiranana Prov., 30.IX.1999), MRSN A4472 (Antsarahana'Ambararato, MP, Antsiranana Prov., 29 XI.1999), MRSN A4456 (Behanjada, MP, Antsiranana Prov., 17.XI.1998); MRSN A4480 (Menamalona, MP, Antsiranana Prov., 11 XII.1999), MRSN A4481 (Menamalona, MP, Antsiranana Prov., 15 XII.1999), MRSN A4482 (Menamalona, MP, Antsiranana Prov., 11.XII.1999); MRSN A4503 (Menamalona, MP, Antsiranana Prov., 15.XII.1999)

Mantella pulchra Parker, 1925. PBZT unlabelled, Fierenana, Toamasina Prov.; MRSN A3060-3062 (Corridor between Moramanga and Zahamena, Tamatave Prov., 10 I.1999); MRSN A2753, 2754 (An'Ala, Tamatave Prov., 1 III 2003), MRSN A59(2-4) (An'Ala, Tamatave Prov., 4.I.1992), MRSN A4487, A4489 (Andasibe surroundings, Tamatave Prov., 1.I.1995).

Mantella viridis Pintak & Böhme, 1988. - MRSN A5055, A5073, A5077 (Ambovomany, AA, Antsiranana Prov., 15 I.2005), MRSN A5102, A5112 (Ambodimanga, AA, Antsiranana Prov., 17.I.2005), MRSN A5064, A5070, A5076, A5094 (Andamanga, AA, Antsiranana Prov., 4.I.2005); MRSN A5075, A5080-5081, A5095, A5097, A5106 (Andronotsimety Ambiney River, AA, Antsiranana Prov., 8 I.2005), MRSN A5095, A5117 (Andohenimangoko, AA, Antsiranana Prov., 15.I.2005); MRSN A5056 (Anketrabe, AA, Antsiranana Prov., 6.I.2005), MRSN A5066 (Anosiravo, AA, Antsiranana Prov., 24 I.2005), MRSN A5088 (Antonboko, AA, Antsiranana Prov., 6 I.2005); MRSN A5054, A5096 (Anjanaharibe, AA, Antsiranana Prov., 8 I.2005); MRSN A5050, A5052, A5067 (Antonboko, AA, Antsiranana Prov., 21 I.2005), MRSN A5115 (La Mere Rouge, AA, Antsiranana Prov., 7.I.2005); MRSN A5113-5114, A5124, A5127 (Maleja, AA, Antsiranana Prov., 7 I.2005); MRSN A5063, A5121 (Mahatsinjo, AA, Antsiranana Prov., 17.I.2005), MRSN A5107 (Porchuite, AA, Antsiranana Prov., 8 I.2005); MRSN A5060 (Tsimanankaratre, AA, Antsiranana Prov., 8 I.2005), MRSN A5082 (Tegnan Antshampano, AA, Antsiranana Prov., 8.I.2005), MRSN A5100 (Tsimanankaratre, AA, Antsiranana Prov., 8.I.2005)